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RAC1 and Melanoma

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Abstract

The mutant *RAC1*^{P29S}, a GTPase member of the RAS superfamily, was identified as the third most common recurrent mutation in melanomas. There is limited information regarding the functional role of *RAC1*^{P29S} as a “driver” in melanogenesis and as a cause for drug resistance. This commentary describes the latest data and provides evidence that supports the notion that *RAC1* is a good drug target to inhibit some melanomas that carry the mutation, but its role in conferring resistance to targeted BRAF or MEK inhibition is still in question.

RAC1 is a GTPase, member of small GTP-binding proteins of the RAS superfamily. The discovery of hotspot mutation in *RAC1* revealed by next-generation sequencing of the coding regions of melanoma (1, 2) provided evidence for a new ‘player’ in melanoma genesis. The recurrent P29S mutation, i.e., substitution of proline at position 29 with serine, is the 3rd most frequent activating mutation in sun-exposed melanoma, occurring at about 4–7% of the patients. It is highly significant, because for the past several years the only two most well characterized hotspot mutations in melanoma were *BRAF*^{V600} (~40%) and *NRAS*^{Q61} (~20%).

There are several novel aspects about *RAC1*^{P29S} in melanoma. Unlike *BRAF*^{V600} and *NRAS*^{Q61}, it carries the characteristic signature for UVB induced DNA damage, a C to T transition in dipyrimidine, CCT>TCT, validating the critical role of sunlight in the onset of melanoma. Indeed, *RAC1*^{P29S} is restricted to hair-bearing cutaneous melanoma, and most of the primary melanoma lesions in the Yale cohort are from sun-exposed areas, and most patients reported excess sun exposure. Unlike *BRAF*^{V600} and *NRAS*^{Q61}, the mutation is not present in benign nevi. In the original Yale cohort, it was predominant in males (95%), but additional studies showed only a slight increase in frequency in males over females (59%) (3).

Another important observation is that, like in *BRAF*^{V600} and *NRAS*^{Q61}, the mutation confers constitutive activity to *RAC1*. This is of critical importance because it constitutes an “oncogene” driver- type of mutation. The sequencing of over 600 sun-exposed melanoma (ours, the Broad Institute and the TCGA) showed that the only other activating mutations (in addition to the BRAF and NRAS mentioned above) is the *MAP2K1*^{P124S} (~2.8%). This is not to diminish the importance of oncogenic mutations in *c-KIT* in mucosal and acral (10–20%), and in *GNA11/GNAQ* in uveal (~80%) melanomas, which are very rare in sun-exposed melanomas.

RAC1, like RAS, cycles between the GTP (active) and GDP (inactive) forms. Our functional studies showed that unlike the RAS mutations, *RAC1*^{P29S} maintains its normal

GTPase activity but displays an increased inherent GDP→GTP nucleotide exchange, which keeps it mostly in the active form (4). When mutant *RAC1*^{P29S} is introduced into normal melanocytes, it increases membrane ruffling, promotes proliferation and migration (1). Biochemical studies showed that *RAC1*^{P29S} maintains an increased binding to target proteins PAK1 and MAP3K11 (MLK3), likely to affect downstream molecular events. The general notion is that *RAC1*^{P29S} has a particular role in early transformation, enhancing cell migration and proliferation, as supported by its early presence in primary melanoma (3).

One of the main goals of cancer sequencing efforts is to identify new targets for therapy as well as new changes that confer drug-resistance and/or alters patients' response to therapy, and *RAC1*^{P29S} is no exception. We validated the role of *RAC1* in melanoma cell proliferation by knockdown experiments with *RAC1* specific shRNA (Figure 1, our unpublished results). The data show downregulation of *RAC1* reduced the rate of cell proliferation of *RAC1*^{P29S} mutant melanoma (such as YUHEF), as well as *RAC1* wild type melanomas (such as YUROL and YUROB), with less of an effect on cells bearing the *BRAF*^{V600} mutation (Figure 1B, C). These data suggest that *RAC1* is a good target for therapy, because it is active also in *RAC1* WT melanomas by other molecular events. These can be changes in the activity of upstream regulators, especially GEFs and GAPs, i.e., enzymes that convert the inactive GDP to the active GTP state, and the GTP back to GDP-state, respectively (5). Unfortunately, the currently available drug that inhibits *RAC1* (EHT1864) is not efficient at inhibiting *RAC1*^{P29S} mutant or wild type melanoma cells (IC₅₀ 4–10 μM), although an inhibitor of its downstream target PAK (PF8055), is relatively potent (IC₅₀ 0.23–1 μM) (our unpublished results). Altogether, our experience show that *RAC1*, like RAS, is a target that is difficult to inhibit with small molecules. However, with the NCI new initiative to identify novel ways to inhibit RAS (5), there is still a possibility that new agents and methods will be developed.

The other major question is whether *RAC1*^{P29S} confers resistance to drugs that are currently being used to target *BRAF*^{V600} mutant cancer cells, including melanomas. This possibility was recently suggested by Watson *et al* (6). The investigators describe resistance to *BRAF* inhibitors (vemurafenib and dabrafenib) and MEK inhibitors (trametinib and PD325901) of two patient-derived *RAC1*^{P29S} mutant melanomas that also carry the *BRAF*^{V600K} or the *NRAS* Q61K mutations, and by overexpressing *RAC1*^{P29S} in melanoma cells. However, melanoma cells carry a large number of mutations and genomic alterations that are likely to affect their response to drugs, and two examples of melanoma are not sufficient to draw conclusion. We have recently performed drug response analyses on ~50 patient-derived early passage melanoma cells focusing on vemurafenib (originally known as PLX4032) and the MEK inhibitor selumetinib (AZD6244) currently used to treat melanoma patients. These melanoma cells are well characterized by whole exome-capture sequencing, so the presence of mutations, deletions/amplification and other genomic aberrations can be easily related to sensitivity and resistance to drugs. The results show that double-mutant *BRAF*^{V600E}/*RAC1*^{P29S} melanoma cells (YURIF) are in the mid-range of sensitivity to vemurafenib (IC₅₀ 0.25 μM) when compared to other *BRAF* mutant cells, where the range is 0.06–0.45 μM (7). On the other hand, these melanoma cells are highly sensitive to selumetinib, IC₅₀ 0.014 μM. In contrast, another *RAC1*^{P29S} melanoma cell line (YUHEF, *BRAF*/*NRAS* WT, see Figure

1) is highly resistant to selumetinib, IC₅₀ of over 10 μM. However, it is not yet clear whether the resistance to the MEK inhibitor is due to the presence of RAC1^{P29S}, or to the loss of NF1 expression we observed in these cells. Loss of NF1 and the presence of RAC1^{P29S}, do not confer resistance to the MEK inhibitors in another melanoma cell line that is BRAF/NRAS WT, and which displays IC₅₀ 0.025 μM. These data show that one cannot predict sensitivity or resistance to BRAF or MEK inhibitors by the presence of activated RAC1 or loss of NF1. More is needed to decipher the relevant molecular signature and markers that can guide us in pursue of precision medicine so patients are selected correctly to targeted therapy.

The unbiased sequencing of the whole-exome show that melanoma is one of the cancers with the highest number of mutations/tumor. Nevertheless, very few “oncogenes”, i.e., genes with activating mutations that can confer advantage to cancer cells and serve as targets, were identified so far (as mentioned above). In contrast, the number of “driver genes” in the tumor-suppressor category is much larger (such as NF1, TP53, CDKN2A, PTEN, PPP6C, RB1, ARID1A, ARID2, to mention the very top on the list). Unfortunately, it is more difficult to restore the function of tumor suppressors as an approach in cancer therapy. It is possible that their downstream target and/or the pathway that they affect can be a subject of further analyses, as for example the effect of PPP6C on Aurora kinase (8, 9). In additions, the mutations in melanoma likely contribute to the immunogenicity of this cancer and can be harnessed in immunotherapy (10, 11), raising the possibility that RAC1^{P29S} is one such antigen that can be explored.

Altogether, we are in a new era in which next-generation sequencing of large number of tumors provided us already with a deep knowledge of the molecular changes in tumors. We are all aware that a single mutation, such as in BRAF, NRAS, RAC1, NF1 or any of the other ‘driver’ genes does not lead to cancer. There is a need to decipher the contribution of the additional changes, and combination of changes, so we can understand the cancer biology and responses to drugs. The development of bioinformatic algorithm that can take into account these changes is critical so we can move to the next step, i.e., apply “precision medicine” to the clinic to optimize the selection of individual patients to targeted and/or immunotherapy.

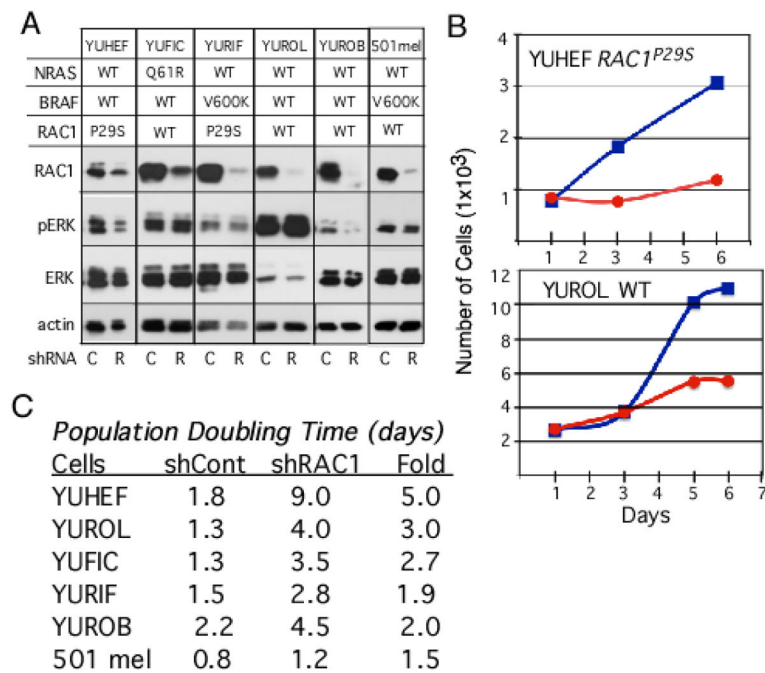
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**Figure 1.**

The consequences of RAC1 knockdown in melanoma cell signaling and proliferation. A. RAC1 and ERK (Extracellular regulated Kinase) in melanoma cells infected with control (C) or RAC1 specific (R) shRNA lentiviral particles. Cells were tested after selection in puromycin (1 μ g/ml) for 10 days. Cell lysates were subjected to Western blot analysis by probing with antibodies against RAC1, phospho-ERK1/2 Thr202/Tyr204 mAb (pERK), ERK1/2 (ERK), and actin as a control. The data show that downregulation of RAC can reduce ERK signaling as shown for YUHEF and YUROB, but not in the other cell lines. B. Cell proliferation in response to RAC1 knockdown. Cell proliferation monitored by counting the number of cells seeded in triplicate wells at 2–3 days intervals in medium containing puromycin; Blue and red curves indicate cells infected with control or RAC1 specific shRNA, respectively. C. Summary of melanoma cells response to RAC1 knockdown. Population-doubling time was calculated from the linear curves as shown in B.